Taxon-specific $\delta^{13}C$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden

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Abstract

Taxon-specific stable carbon isotope ($\delta^{13}C$) analysis of chitinous remains of invertebrates can provide valuable information about the carbon sources used by invertebrates living in specific habitats of lake ecosystems (for example, sediments, water column, or aquatic vegetation). This is complementary to $\delta^{13}C$ of sedimentary organic matter (SOM), which provides an integrated signal of organic matter produced in a lake and its catchment, and of diagenetic processes within sediments. In a sediment record from Strandsjön (Sweden) covering the past circa 140 years, we analyzed SOM geochemistry ($\delta^{13}C$, $C:N_{atomic}$, organic carbon content) and $\delta^{13}C$ of chitinous invertebrate remains in order to examine whether taxon-specific $\delta^{13}C$

records could be developed for different invertebrate groups and whether these analyses provide insights into past changes of organic carbon sources for lacustrine invertebrates available in benthic and planktonic compartments of the lake.

Invertebrate taxa included benthic chironomids (*Chironomus*, Chironomini excluding *Chironomus*, Tanytarsini, and Tanypodinae), filter-feeders on suspended particulate organic matter (*Daphnia*, *Plumatella* and *Cristatella mucedo*), and Rhabdocoela. $\delta^{13}C$ of chironomid remains indicated periodic availability of ^{13}C-depleted carbon sources in the benthic environment of the lake as $\delta^{13}C$ values of the different chironomid taxa fluctuated simultaneously between -34.7 and -30.5‰ (VPDB). *Daphnia* and Bryozoa showed parallel changes in their $\delta^{13}C$ values which did not coincide with variations in $\delta^{13}C$ of chironomids, though, and a 2-3‰ decrease since circa AD 1960. The decrease in $\delta^{13}C$ of *Daphnia* and Bryozoa could indicate a decrease in phytoplankton $\delta^{13}C$ as a result of lower lake productivity, which is in accordance with historical information about the lake that suggests a shift to less eutrophic conditions after AD 1960. In contrast, Rhabdocoela cocoons were characterized by relatively high $\delta^{13}C$ values (-30.4 to -28.2‰) that did not show a strong temporal trend, which could be related to the predatory feeding mode and wide prey spectrum of this organism group. The taxon-specific $\delta^{13}C$ analyses of invertebrate remains indicated that different carbon sources were available for the benthic chironomid larvae than for the filter-feeding *Daphnia* and bryozoans. Our results therefore demonstrate that taxon-specific analysis of $\delta^{13}C$ of organic invertebrate remains can provide complementary information to measurements on bulk SOM and that $\delta^{13}C$ of invertebrate remains may allow the reconstruction of past changes in carbon sources and their $\delta^{13}C$ in different habitats of lake ecosystems.

Keywords: invertebrates, chitinous remains, lake sediment, stable carbon isotopes, sedimentary organic matter

Introduction

The stable carbon isotope composition of bulk organic matter in lake sediments is determined by the $\delta^{13}C$ values of different organic matter sources available in a lake and its catchment and by the preservation of the various inputs (Meyers and Teranes 2001). Environmental changes can affect the isotopic composition of organic matter, the amount of organic matter produced within lakes, the relative contribution of autochtonous and allochthonous organic matter, and the transport processes leading to the incorporation of organic matter into lake sediments. Therefore, analysis of $\delta^{13}C$ of bulk sedimentary organic matter (SOM) can potentially provide information on past changes in climate, productivity, origin of organic matter, pollution, and in-lake carbon cycling (Leavitt et al. 2006; Leng et al. 2005; Meyers and Lallier-Vergès 1999). It is difficult, however, to reconstruct limnological changes in different compartments (benthic, pelagic) of a lake based on $\delta^{13}C$ of SOM only, since variations in $\delta^{13}C$ of SOM may reflect variations in the isotopic composition of organic matter within the lake as well as in the lake catchment. Even if the approach is applied to lakes in which SOM originates mainly from lacustrine sources, variations in bulk SOM isotopic composition may reflect changes in carbon sources and organic matter production in the littoral, pelagial or profundal of the lake and selective preservation/degradation of various compounds and tissues. A possible approach to circumvent this problem is to analyze organic remains of selected organisms

originating from the lake itself. If these remains can be identified and associated with organism groups with clear habitat preferences it may be possible to determine past changes in the carbon isotopic composition of aquatic organic matter in specific habitats and compartments of lakes.

Recently, a number of studies analyzed $\delta^{13}C$ of specific organic remains that preserve well in lake sediments. These include remains of primary producers such as *Potamogeton* (Herzschuh et al. 2010; Turney 1999) and traces of organic matter within diatom valves (Barker et al. 2013; Hurrell et al. 2011) as well as of secondary producers such as aquatic invertebrates (for example, cladocerans (Perga 2010) and chironomids (Wooller et al. 2008)). Chitinous invertebrate remains largely consist of proteins and chitin and are chemically robust and relatively resistant to microbial degradation, especially if buried under anoxic conditions (Verbruggen et al. 2010). They can be identified and associated with a particular group of organisms. Therefore, it can be ensured that the analysed remains originate from the lake itself. Since different invertebrate groups are characterized by different feeding modes (for example, filter-feeders, deposit-feeders, or predators) and habitat preferences (planktonic or benthic, littoral or profundal), taxon-specific analyses of invertebrate $\delta^{13}C$ can potentially provide insights on processes that are active in different compartments of lakes and affect the lacustrine food web and carbon cycle.

Until very recently chitinous aquatic invertebrate remains have received little attention with regard to carbon isotope analysis. Perga (2010) demonstrated that strong correlations exist between $\delta^{13}C$ values of tissue of living cladocerans of the genus *Bosmina* and *Daphnia* and their chitinous carapaces. However, she concluded that taphonomic processes should nevertheless be taken into account when interpreting palaeoecological records as they can cause lower $\delta^{13}C$ (<1‰) of *Daphnia*

remains, because isotopically light C compounds seem to be selectively removed by microbial degradation (Perga 2011). Wooller et al. (2008) were the first to analyze $\delta^{13}C$ of head capsules of chironomid larvae in a sediment sequence from an Icelandic lake. They reported similar patterns in stable isotope values of chironomids and SOM. Van Hardenbroek et al. (2010a) studied the effects of various chemical pre-treatments commonly used in palaeoelimnological studies on carbon isotopic measurements on chitinous remains of chironomid larvae and showed that these treatments had little effect on the $\delta^{13}C$ of the larval head capsules. These authors also demonstrated that head capsule $\delta^{13}C$ is influenced by the chironomid diet and that methanogenic carbon can influence the carbon isotopic signature of chironomid exoskeletons. Heiri et al. (2012) and Frossard et al. (2013) demonstrated that $\delta^{13}C$ values of chironomid head capsules are closely related to the carbon isotopic composition of the larval body and the food ingested by chironomid larvae.

Only very few studies are available that examine $\delta^{13}C$ of remains of multiple invertebrate groups in the same sediment samples. Van Hardenbroek et al. (2012; 2013) examined the relationship of $\delta^{13}C$ values of remains of five chironomid taxa and the resting eggs (ephippia) of water fleas of the genus *Daphnia* with methane fluxes in 17 lakes in Sweden and Siberia and reported a statistically significant relationship between diffusive methane flux measured at the lake surface and $\delta^{13}C$ of the fossils of some chironomid groups and of *Daphnia* ephippia. Wooller et al. (2012) and van Hardenbroek et al. (2013) presented down-core records of chironomid and *Daphnia* $\delta^{13}C$ from thermokarst lakes in Alaska and Siberia, respectively, which suggest major changes in the carbon cycle and methane availability in these lakes. Similar results were obtained by Frossard et al. (2014) using sediment cores from Lake Annecy (France) in which they observed decreasing $\delta^{13}C$ values in remains of

pelagic Cladocera and three chironomid taxa during 1930s -1950s. Frossard et al. (2014) suggest that eutrophication and increased hypoxia led to increased incorporation of ^{13}C-depleted carbon (microbially respired and/or methane-derived) into the invertebrate tissues, which explains the lower $\delta^{13}C$ values in the invertebrate remains.

Down-core studies examining $\delta^{13}C$ in the remains of multiple planktonic and benthic invertebrate groups such as cladocerans, bryozoans, chironomids, and other aquatic insects allows the taxon-specific records to be compared among indicator groups in order to detect variations in $\delta^{13}C$ common to several groups. Hence, past changes in the carbon cycle of lakes tracked by different invertebrates originating from the benthic or pelagic part of lakes could be separated from background variations in $\delta^{13}C$ common to all indicators. Here we present a first study presenting $\delta^{13}C$ records based not only on the chitinous remains of Chironomidae (non-biting midges) and Cladocera (water fleas), but also on the remains of Bryozoa (moss animals), and Rhabdocoela (Turbellaria: free-living flatworms). Analyses were made on sediments collected from Strandsjön, a small shallow lake in South-central Sweden (59°52'28" N and 17°10'5" E, 51 m a.s.l.). The catchment contains pine forests and pastures, some with dairy farming since the beginning of the 20th century that led to increased nutrient loading of the lake until the installation of waste water treatment in the 1960s (Brunberg and Blomqvist 1998) and TP values were lowered to 41.3 µg L^{-1} in 2008 (van Hardenbroek et al. 2012). The history of eutrophication and recovery of Strandsjön was expected to have affected $\delta^{13}C$ values of algae, as these are usually ^{13}C-enriched when productivity is higher (Brenner et al. 1993; Hollander et al., 1993). We therefore expected productivity-related changes in algal $\delta^{13}C$ to be reflected in $\delta^{13}C$ of algivorous filter-feeders like *Daphnia* and Bryozoa and their remains. In

contrast, we expected that $\delta^{13}C$ values would be not (or less clearly) related to past changes in productivity for predatory Rhabdocoela or for benthic chironomids, the latter feeding on a mixture of detritus from the water column and decomposed SOM. Strandsjön was selected because $\delta^{13}C$ of invertebrate remains in surface sediments from this lake were previously analysed by van Hardenbroek et al. (2012), providing additional information for the interpretation of invertebrate $\delta^{13}C$ in this down core study.

Materials and Methods

Sediment characteristics

The coring location was chosen at 2.4 m water depth (Fig. 1). This location represents the deepest part of a transect of surface sediment samples obtained from the lake in which $\delta^{13}C$ of invertebrate remains were analyzed (van Hardenbroek et al. 2012). A 36 cm long core with undisturbed sediment-water interface was obtained using a gravity corer (Uwitec, Austria) and sub-sampled in the field at 1 cm resolution. The samples were stored in plastic bags and kept cool and dark until freeze-drying upon arrival in the laboratory. Freeze-dried samples were used for dating by gamma spectrometry using a Canberra low-background Ge-well detector. ^{210}Pb was measured via its gamma-peak at 46.5 keV, ^{226}Ra via the granddaughter ^{214}Pb (peaks at 295 and 352 keV), and ^{137}Cs via its peak at 661 keV.

Samples for stable isotope analysis of SOM were rinsed in 2.5% HCl for 15 minutes to remove carbonates, rinsed three times with demineralized water,

centrifuged 4 min at 2000 rpm to remove excess water, and freeze-dried. $C:N_{atomic}$ ratios, as well as stable carbon isotopes of SOM were analyzed on a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 IRMS. Two laboratory standards (nylon and peach leaves) of known relation to international standards for VPDB were used as references. Replicate sample measurements on standards (n = 43) indicated an analytical error (2σ) of ±0.05‰ for $\delta^{13}C$.

$\delta^{13}C$ of invertebrate remains

Samples for $\delta^{13}C$ analysis of chitinous invertebrate remains were deflocculated in 10% KOH for 2 h at room temperature and sieved with 200- and 100-µm sieves (van Hardenbroek et al. 2010b). Rinsing with an alkaline solution has the added advantage of removing adsorbed fulvic and humic acids (Stevenson 1982). Sieve residues were soaked in 2.5% HCl for 15 minutes, rinsed three times and stored in demineralized water in the dark. Remains were identified under a dissecting microscope at 40–100x magnification following Wood and Okamura (2005) for Bryozoa statoblasts, Vanderkerkhove et al. (2004) for the resting stages of *Daphnia*, Brooks et al. (2007) for chironomid head capsules, and Luther (1955) and Frey (1964) for Rhabdocoela cocoons. Chironomid head capsules were separated into the categories *Chironomus* spp., Chironomini (excluding *Chironomus*), Tanytarsini, Orthocladiinae, and Tanypodinae. Furthermore, we differentiated ephippia of the cladoceran genus *Daphnia*, statoblasts of the Bryozoa taxa *Plumatella* and *Cristatella mucedo*, and the chitinous cocoons of Rhabdocoela.

After identification, remains were transferred with forceps directly into pre-weighed ultraclean tin cups. Tin cups were dried on a hotplate at 50°C for 24 h after

which they were re-weighed and crimped for stable isotope analysis. The average number of remains in a sample was 18 for *Chironomus* (average sample weight 23 μg), 36 for other Chironomini (33 μg), 45 for Tanytarsini (24 μg), 22 for Tanypodinae (22 μg), 34 for *Daphnia* (40 μg), 41 for *Plumatella* (18 μg), 1.2 for *C. mucedo* (28 μg), and 40 for Rhabocoela (47 μg) (Supplementary Table S1). Control samples of water from sieve residues were evaporated in tin cups and no carbon contamination was detected. Samples of invertebrate remains were analyzed on a Fisons NA 1500 NCS Elemental Analyzer coupled to a Thermo Electron Delta plus IRMS. A laboratory standard (Naxos GQ graphite quartzite) of known relation to international standards for VPDB was used as reference. Replicate sample measurements of the secondary standard (n = 30) indicated an analytical error (2σ) of ±0.05‰.

Results

Age-depth model

High ^{137}Cs-activity (>2000 Bq kg^{-1}) was measured above 4.5 cm sediment depth in the Strandsjön sediments pointing to a link to the 1986 Chernobyl accident for this material. This suggests that the sediment at 4.5 cm depth was deposited in 1986 or shortly thereafter. ^{137}Cs-profiles measured in European lake sediments typically feature a second maximum at AD 1963, coinciding with the peak in above-ground nuclear bomb testing (Appleby 2001), but in Strandsjön this feature seems to be hidden by slight downward penetration of the high ^{137}Cs-activity in the top 4.5 cm. We measured surface contents of unsupported ^{210}Pb of approximately 220 Bq kg^{-1}. ^{210}Pb contents decreased more or less exponentially down core with the exception of

two outliers at 17-18 and 25-26 cm depth. The calculated flux of unsupported ^{210}Pb is 62 Bq m^{-2} y^{-1}, which is similar to the supply rate of Knud Sø in Denmark (58 Bq m^{-2} y^{-1}) presented by Appleby (2001). Age-depth modeling using ^{210}Pb was based on a modified constant rate of supply (CRS) model (Appleby and Oldfield 1978) with 4.5 cm fixed at 1986 as a reference point (Fig. 2). The inventory below 25 cm was calculated on the basis of a regression of the content of unsupported ^{210}Pb versus accumulated mass above this level. The age-model was extrapolated for samples below 31 cm, resulting in an estimated age for the lowermost sample of circa AD 1870 ±15 years. Due to the outliers in the ^{210}Pb measurements, we consider the CRS model less reliable in the deeper section of the core, which is why we report approximate ages for the record.

Sediment geochemistry and δ^{13}C of invertebrate remains

SOM δ^{13}C decreased from approximately -30 to -33‰ throughout the core and the C:N_{atomic} ratio showed a small, gradual decrease from approximately 11 to 9 (Fig. 3). The carbon content of the sediment was 15-20 % by weight (wt.%) in the lower part of the record up to 15 cm depth, then decreased to 10.5 wt.% at 10 cm depth, and increased again to 12.6 wt.% in the top 7 cm.

δ^{13}C values of filter-feeders that retrieve their food directly from the water column (*Daphnia, C. mucedo,* and *Plumatella*) remained relatively stable for much of the record (approximately 12-35 cm depth). Their δ^{13}C then decreased by 2-3‰ between approximately 5 and 10 cm depth (AD ~1960 to 1980), and increased again by approximately 1-1.5‰ in the uppermost sediments. *C. mucedo* had lowest δ^{13}C values (-35.1 to -31.2‰), followed by *Daphnia* (-32.8 to -30.5‰) and *Plumatella* (-

33.1 to -28.0‰) (Fig. 4). $\delta^{13}C$ of larval remains of chironomids showed variations at a higher frequency ranging between -34.6‰ and -30.3‰ (Fig. 3). Lowest values were recorded by *Chironomus* (-34.6 to -30.8‰), followed by Tanytarsini (-33.9 to 30.3‰), Tanypodinae (-33.8 to -31.0‰) and Chironomini other than *Chironomus* (-33.5 to -30.5‰) (Fig. 4). For most chironomid groups, relative shifts in $\delta^{13}C$ values were very similar with local minima in $\delta^{13}C$ recorded at 27.5, 20.5, 12.5, and 2.5-4.5 cm sediment depth (AD ~1890, ~1920, ~1950, and ~1990, respectively). The exception are the remains of *Chironomus*, which are present in the sediments between 0-19 cm and at 32 cm depth, as they show higher amplitude variations than the other chironomid groups. $\delta^{13}C$ of Rhabdocoela cocoons showed less temporal variability and was around -29.5‰ (-30.2 to -28.4‰) throughout the core.

Discussion

Feeding ecology of invertebrate groups

For the interpretation of $\delta^{13}C$ values it is important to understand the feeding ecology and habitat of the invertebrates in this study. The larvae of Chironomidae are benthic and, in lakes, live in or on sediments, or on hard substrates such as rocks, submerged wood and aquatic macrophytes. The Tanytarsini are typically algivores and deposit-feeders with limited capabilities for burrowing into sediments (Merritt et al. 2008; Mihuc and Toetz 1994; Vander Zanden and Rasmussen 1999). The Chironomini include a number of taxa that tend to burrow deeper into sediments, or in some cases into aquatic macrophytes. They feed predominantly as deposit-feeders, although some groups also filter particles from water which is pumped through the tubes the larvae

live in (Merritt et al. 2008; Moller Pillot 2009; Moog 2002), a habitat where they can also access ^{13}C-depleted biomass of methane oxidizing bacteria (Jones et al. 2008). The Tanypodinae are mainly free-living and mobile predators. However, several Tanypodinae are known to also feed on the substrate the larvae live on (Merritt et al. 2008; Vallenduuk and Moller Pillot 2007). Bryozoa live predominantly as colonies attached to hard substrates and feed on suspended nanoplanktonic algae from the water column, but they cannot move through the water themselves (Kaminski 1984; Okamura and Hatton-Ellis 1995; Wood and Okamura 2005). *Daphnia* are mobile zooplankton feeding on fine particles in the water column that range between 0.5 to 30 µm in diameter and include phytoplankton and bacteria (Geller & Müller 1981). It can be assumed, therefore, that $\delta^{13}C$ of *Daphnia* and Bryozoa reflects the material they filter from the water column. Rhabdocoela are active predators feeding on small invertebrates and large ciliate protozoans as well as scavengers (Jennings 1957; Kolasa and Tyler 2010). In contrast to benthic chironomids and filter-feeding *Daphnia* and bryozoa, rhabdocoels are probably less directly affected by changes in $\delta^{13}C$ values of phytoplankton or SOM but have a $\delta^{13}C$ signature representing a mixture of their prey organisms.

Differences in $\delta^{13}C$ offsets between invertebrate taxa

The carbon isotopic composition of invertebrate remains in Strandsjön showed some variability between the different invertebrate groups (Fig. 4). Similar to the findings of van Hardenbroek et al. (2013), the remains of *Chironomus* were ^{13}C-depleted relative to other chironomids and invertebrate groups. In Strandsjön, $\delta^{13}C$ values of *Chironomus* head capsules were only slightly more negative than the remains of other

Chironomidae and of *Daphnia*, which in turn tended to be only slightly more negative than the $\delta^{13}C$ values recorded for bulk SOM. Interestingly, $\delta^{13}C$ values recorded for statoblasts of the bryozoan *C. mucedo* were more negative than those of chironomid remains in most samples, whereas the remains of *Plumatella* and of Rhabdocoela cocoons were distinctly ^{13}C-enriched relatively to SOM and other invertebrates. We found no published $\delta^{13}C$ values of sedimentary remains of *Plumatella* and Rhabdocoela and only one published study with few $\delta^{13}C$ measurements on *C. mucedo* from two sediment records in the United Kingdom (Turney, 1999). In this study four $\delta^{13}C$ values of *C. mucedo* statoblasts from Llanilid are presented, ranging between -29 and -25‰, approximately 0-1‰ lower than $\delta^{13}C$ values of SOM. For the second site, Gransmoor, two $\delta^{13}C$ values of *C. mucedo* statoblasts are between -24 and -22‰ and approximately 0-4 ‰ higher than $\delta^{13}C$ of SOM. The variability in $\delta^{13}C$ offset between *C. mucedo* and SOM and the lack of stable isotope data clearly indicates more work is required to understand which carbon sources are used by Bryozoa and Rhabdocoela before their $\delta^{13}C$ values can be interpreted conclusively.

We expected that $\delta^{13}C$ offsets between taxa largely reflect differences in stable isotope composition of their food sources. It must be considered, however, that biochemical processes during the synthesis of the body tissues may also potentially influence invertebrate $\delta^{13}C$ and can play a role when comparing remains from different invertebrate taxa. Generally, the $\delta^{13}C$ of chitinous invertebrate structures is closely related to the carbon isotopic composition of the soft tissue of the invertebrates in question and of the food available for the animals (DeNiro and Epstein 1978; Rau 1980). Schimmelmann (2011) reviewed the isotopic difference for eight invertebrate-diet combinations and found that the isotopic difference between diet and the corresponding D-glucosamine hydrochloride from invertebrate chitin

ranges between −2.2 and +2.1‰ (average 0.0 ± 1.5‰). His results suggest that taxa can differ in the offset between $\delta^{13}C$ of ingested carbon and $\delta^{13}C$ of their chitinous structures, but that this variation is mostly limited to approximately 0-2‰. Experiments with laboratory cultures of *Chironomus riparius* indicated a ^{13}C-depletion of 4th instar larval head capsules compared with 4th instar larval body tissue of 1-2‰ (Heiri et al. 2012) and 0.9 ± 0.2‰ (Frossard et al. 2013). Perga (2011) demonstrated that the offset in $\delta^{13}C$ between whole *Daphnia* and their ephippia collected from the same water sample was within the standard deviation (0.2‰) of replicate $\delta^{13}C$ measurements. Similarly, Macko et al. (1989) reported a 0.4-0.8‰ ^{13}C-depletion of carapaces compared to body tissue of three marine crustaceans. As mentioned before, little to nothing has been published about the stable isotope composition of Bryozoa and Rhabdocoela or their remains. The large difference in $\delta^{13}C$ values of remains of Rhabdocoela and *Plumatella*, relative to other invertebrate remains examined in our record suggests that these taxa were able to access different carbon sources than the other analysed invertebrate taxa. This interpretation is supported by the observation that an offset is apparent even between *Plumatella* and *C. mucedo*, both bryozoans, for which a similar offset between food, body tissue and statoblasts can be expected. In our record *Plumatella* had on average 3.5‰ higher $\delta^{13}C$ than *C. mucedo* and it is possible that this was the result of a difference in the $\delta^{13}C$ of the particles ingested by these two taxa when filtering the water. Kaminski (1984) demonstrates that *C. mucedo* selects small seston (<7 µm in diameter), which can include bacteria, whereas *Plumatella repens* prefers slightly larger particles (ranging from 5 to 17 µm in diameter).

Trends in $\delta^{13}C$ of invertebrate remains

The four chironomid taxa showed similar variations in $\delta^{13}C$ with several minor minima during the past circa 140 years (Fig. 3). All analyzed chironomid groups featured the same type of high frequency signal, which makes it very unlikely that these variations in $\delta^{13}C$ are related to changes in taxonomic composition that happened simultaneously within all four groups. Since *Chironomus*, other Chironomini, and Tanytarsini are largely detritivores and partly grazers and filterers, and Tanypodinae are predators, it can be expected that the $\delta^{13}C$ of the chironomid remains analyzed here mostly reflected the $\delta^{13}C$ of sedimentary detritus and detritivorous prey animals. $\delta^{13}C$ of SOM in the Strandsjön record decreased gradually between 35 and 0 cm depth. However, chironomids did not show the same decreasing trend in $\delta^{13}C$ that we observed for SOM. This suggests that the invertebrates were selectively feeding on and digesting certain organic matter components in the sediments. Wooller et al. (2008; 2012) observed trends in $\delta^{13}C$ of chironomid remains that were roughly similar to the patterns observed for SOM. In these studies, however, Wooller et al. (2008; 2012) did not include taxonomic information about the analyzed chironomids. This makes a direct comparison between their records and our study difficult, since not all chironomid tribes and subfamilies can be expected to have the same $\delta^{13}C$ values (van Hardenbroek et al. 2012), at least not in every lake. In a study examining the sediments of a Siberian thermokarst lake, van Hardenbroek et al. (2013) found that variations in $\delta^{13}C$ of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* did not follow the same pattern as $\delta^{13}C$ of SOM. Only the chironomid subfamily Orthocladiinae was characterized by $\delta^{13}C$ values very similar to those of SOM. This could be explained by a preference of many Orthocladiinae for a diet of periphyton or SOM, whereas the diet of *Chironomus*, Chironomini, Tanytarsini, and

Daphnia consisted, at least in part, of other components including planktonic algae and bacteria. Van Hardenbroek et al. (2012; 2013) suggested that varying proportions of methane oxidizing bacteria (MOB) in the diet of *Chironomus*, Chironomini, Tanytarsini, and *Daphnia* may be at least partly responsible for the difference in $\delta^{13}C$ between the different invertebrate groups examined in their study. Furthermore, Wooller et al. (2012) argued that methane-derived carbon led to lower $\delta^{13}C$ values of chironomids and *Daphnia* in sections of a sediment record from arctic Alaska. Frossard et al. (2014) also suggested that similar decreases of $\delta^{13}C$ values in two Chironomini taxa, Tanypodinae, and pelagic Cladocera in a sediment record Lake Annecy (France) were linked to increased availability of ^{13}C-depleted methane-derived organic carbon. Chironomid larvae feeding partly on MOB can be strongly ^{13}C-depleted, as shown for several species (Jones et al. 2008; Kiyashko et al. 2001; Zemskaya et al. 2012).

For Strandsjön, relatively high $\delta^{13}C$ values for all chironomid taxa and for *Daphnia* suggest that MOB may have been a less relevant food source than in the lakes studied by Wooller et al. (2012), van Hardenbroek et al. (2013), and Frossard et al. (2014). However, the finding that *Chironomus* and *C. mucedo* are relatively ^{13}C-depleted could be explained by a higher importance of MOB in their diet than in the food ingested by the other invertebrate groups. Larvae of several *Chironomus* species have been shown to incorporate carbon originating from methane into their body tissue (Jones et al. 2008). The feeding ecology of *C. mucedo* is still poorly studied, but as a filter-feeder that can ingest particles in the μm range (Kaminski 1984), the species may be able to feed on MOB in the water column. Similarly, the lower $\delta^{13}C$ values of chironomid remains of the groups Chironomini, Tanypodinae, and Tanytarsini around AD ~1890, ~1920, ~1950, and ~1990 could be explained by

increasing importance of MOB in their diet. However, variations in $\delta^{13}C$ of chironomid remains in Strandsjön were muted and well within the range of values expected for photosynthetically produced organic matter (France 1995a,b; Meyers and Teranes 2001; Bade et al. 2006). Therefore, the observed changes in chironomid $\delta^{13}C$ might also be caused by changes in the relative contribution of isotopically different carbon sources, including allochtonous inputs, remains of aquatic macrophytes, benthic algae, and periphyton that all have higher $\delta^{13}C$ values than pelagic algae (France 1995a,b; Meyers and Terranes 2001). Changes in allochtonous inputs are not obvious from the low and relatively stable $C:N_{atomic}$ ratio of SOM that suggests mainly autochthonous carbon sources. Benthic algae are not considered as an important food source, as limited light penetration inhibits their growth in Strandsjön with dissolved organic carbon concentrations as high as 20.8 mg L^{-1} (van Hardenbroek et al. 2012). Finally, it is unlikely that chironomid $\delta^{13}C$ responded to changes in concentration or $\delta^{13}C$ of dissolved inorganic carbon in the water column, as this would lead to changes in $\delta^{13}C$ of pelagic algae as well, and would be expected to be registered also in the $\delta^{13}C$ of the filter-feeding *Daphnia* and bryozoans. Remaining explanations for the observed variations in chironomid $\delta^{13}C$ include changes in the biomass of macrophytes, periphyton, or MOB in Strandsjön which may have affected the importance of these carbon sources in the diet of chironomid larvae.

$\delta^{13}C$ values of the filter-feeding *Daphnia*, *C. mucedo*, and *Plumatella* were relatively constant in the older part of the record compared with chironomids; the filter-feeders had a decreasing trend in $\delta^{13}C$ in the youngest sediments, followed by a slight reversal to higher values in the uppermost samples. This pattern could be related to changes in $\delta^{13}C$ of phytoplankton in the water column, since planktonic algae can be expected to be an important component of the diet of *Daphnia* and

Bryozoa. The observed variations would be easiest to explain by changes in lake productivity, since $\delta^{13}C$ of phytoplankton in productive meso- to eutrophic lakes is often higher than $\delta^{13}C$ of phytoplankton growing in oligotrophic conditions (Brenner et al. 1999; Hollander et al. 1993). In Strandsjön, $\delta^{13}C$ of *Daphnia* and *C. mucedo* showed a maximum at ~AD 1960. $\delta^{13}C$ of *Plumatella* also showed relatively high values in this section of the record, although the maximum was not as pronounced as for the other two taxa and *Plumatella* was already characterized by relatively high $\delta^{13}C$ values in the period preceding ~AD 1960. A peak in lake nutrient concentration during this period agrees with what is known of the trophic state history of the lake: An increase in nutrient input as agriculture developed around the lake led to eutrophication in the 20th century, causing increasingly frequent algal blooms until the installation of a water treatment plant for the inflow in AD 1960, after which the water quality improved (Brunberg and Blomqvist 1998). In contrast to $\delta^{13}C$ of *Daphnia* and bryozoans, $\delta^{13}C$ of SOM did not seem to track the eutrophication history of the lake. This suggested that carbon isotopic analyses of invertebrate remains might be considerably more sensitive to past changes in $\delta^{13}C$ of organic matter produced in the water column than bulk SOM $\delta^{13}C$, at least in relatively small, shallow, and productive lakes such as Strandsjön.

Conclusions

Our results demonstrate that taxon-specific $\delta^{13}C$ records can be developed for a range of chitinous invertebrate remains found in lake sediments. In Strandsjön, our approach revealed a differential response of $\delta^{13}C$ of benthic taxa (chironomids and rhabdocoels) to past environmental change compared with $\delta^{13}C$ of taxa feeding predominantly on

suspended particulate organic matter (*Daphnia* and bryozoans). Hence, our study indicates that $\delta^{13}C$ of chitinous invertebrate remains can provide valuable additional information to $\delta^{13}C$ of SOM, as the latter cannot distinguish between carbon sources in different compartments of a lake. Additional studies relating the stable isotope composition of various aquatic invertebrates to the values of their fossilizing structures, as well as studies establishing the link between $\delta^{13}C$ of the diet and $\delta^{13}C$ of freshwater invertebrate tissues are urgently needed to improve the interpretation of invertebrate $\delta^{13}C$ in sediment records. More studies examining $\delta^{13}C$ of different organic invertebrate remains from the same sediment record are necessary to confirm that $\delta^{13}C$ of phytoplankton can be reliable traced by examining the carbon isotopic composition of the remains of filter-feeding *Daphnia* and Bryozoa, and that such analyses can reveal a different signal than the one captured by SOM or by the remains of zoobenthos. The results from Strandsjön demonstrate the potential of taxon-specific stable isotope analysis of invertebrate remains for environmental reconstructions and provide, to our knowledge, the taxonomically most diverse such study available to date. It is likely that similar taxon-specific records will soon be produced for stable isotopes of other elements (H, O, N, S) that can give further insights in the functioning of aquatic ecosystems over long time scales.

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Figure captions

Fig. 1 Site location and bathymetric map of Strandsjön (redrawn after Brunberg and Blomqvist 1998). Coring site is indicated by X, inlets and outlets are indicated by arrows.

Fig. 2 Activity of ^{210}Pb (left) and ^{137}Cs (middle) and the age model (right) for the sediment record from Strandsjön based on a constant rate of supply model for ^{210}Pb with the 4.5 cm peak in ^{137}Cs activity as reference point for the 1986 Chernobyl accident.

Fig. 3 δ^{13}C values of invertebrate remains and geochemistry of sedimentary organic matter (δ^{13}C, organic carbon content, and C:N_{atomic} ratio) in the sediment record from Strandsjön. Invertebrate taxa are indicated by open triangles (*Chironomus*), open circles (Chironomini), open grey squares (Tanytarsini), open diamonds (Tanypodinae), solid circles (*Daphnia*), solid triangles (*Cristatella mucedo*), solid diamonds (*Plumatella*), and solid grey squares (Rhabdocoela).

Fig. 4 Boxplots with δ^{13}C values of the different invertebrate taxa in this study and of sedimentary organic matter (SOM).

Figure 1
Click here to download Figure: Fig 1 Location.eps

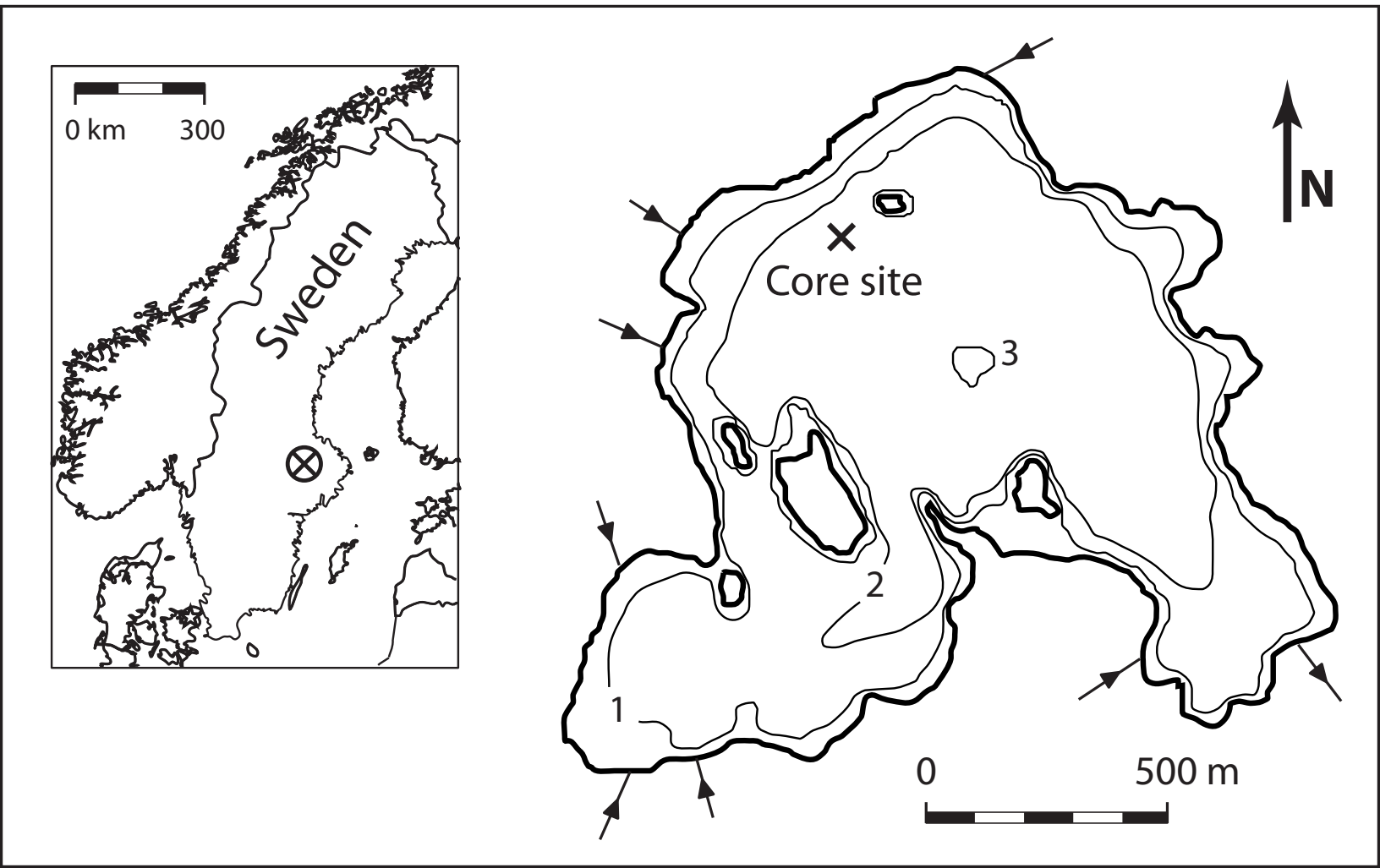


Figure 2
Click here to download Figure: Fig 2 Age depth v2.eps

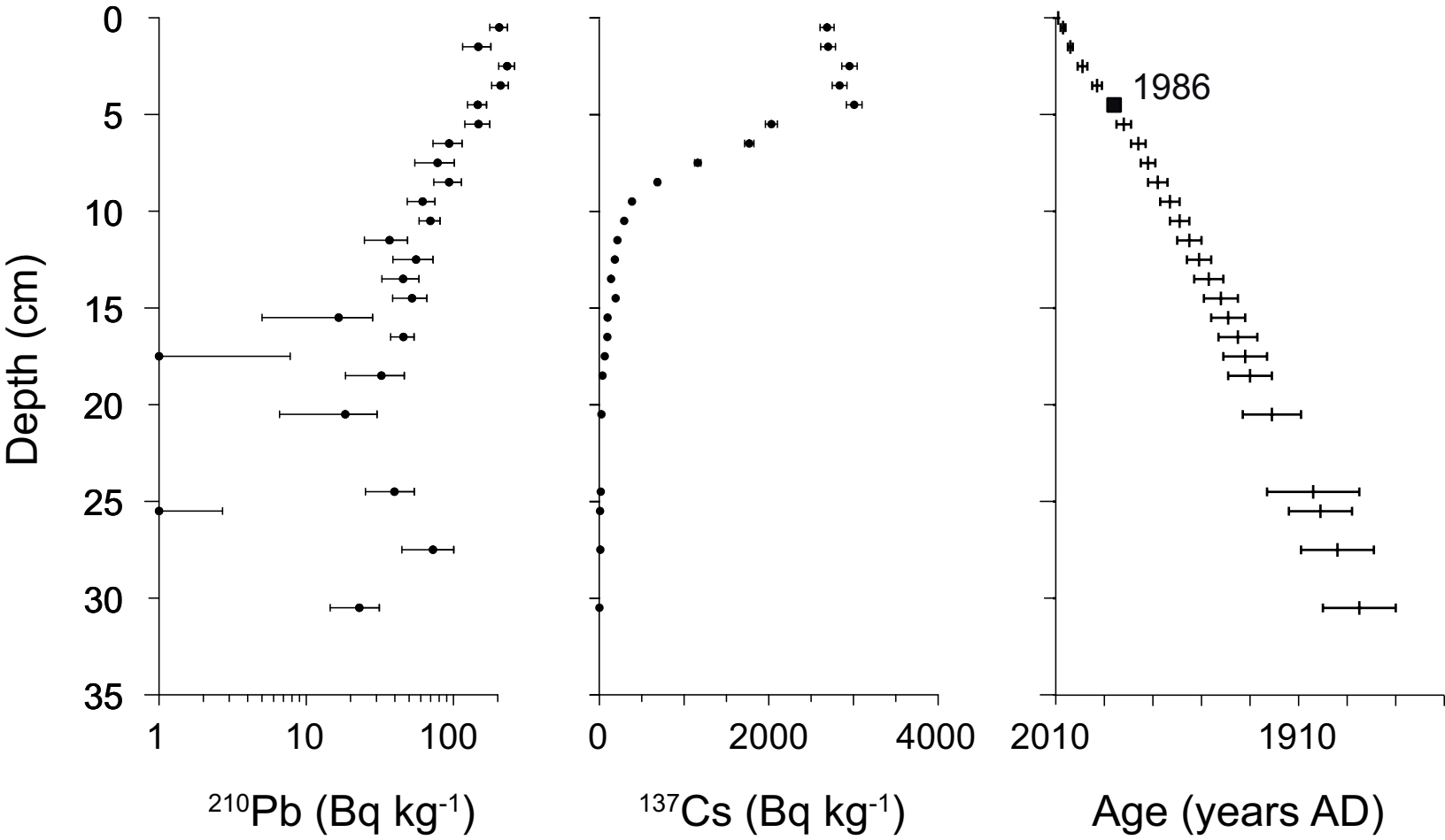


Figure 4
Click here to download Figure: Fig 4 boxplot v2.eps

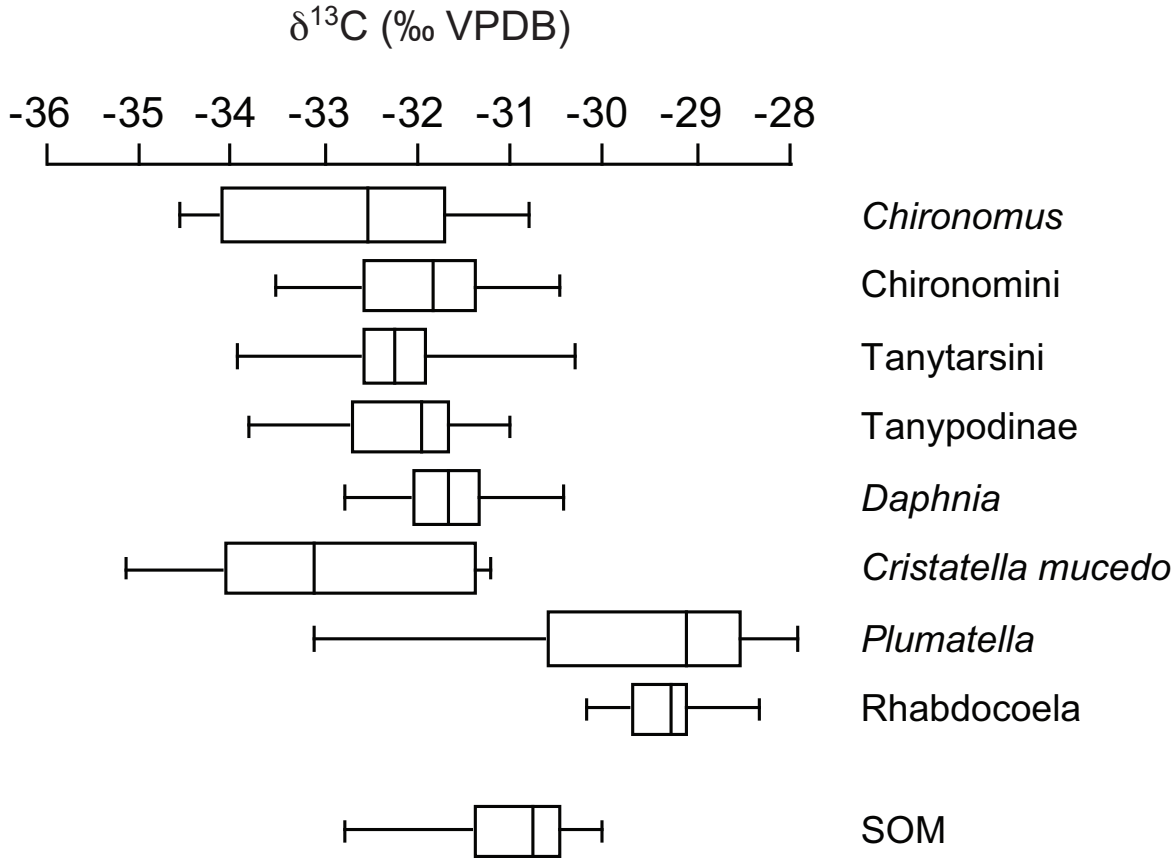
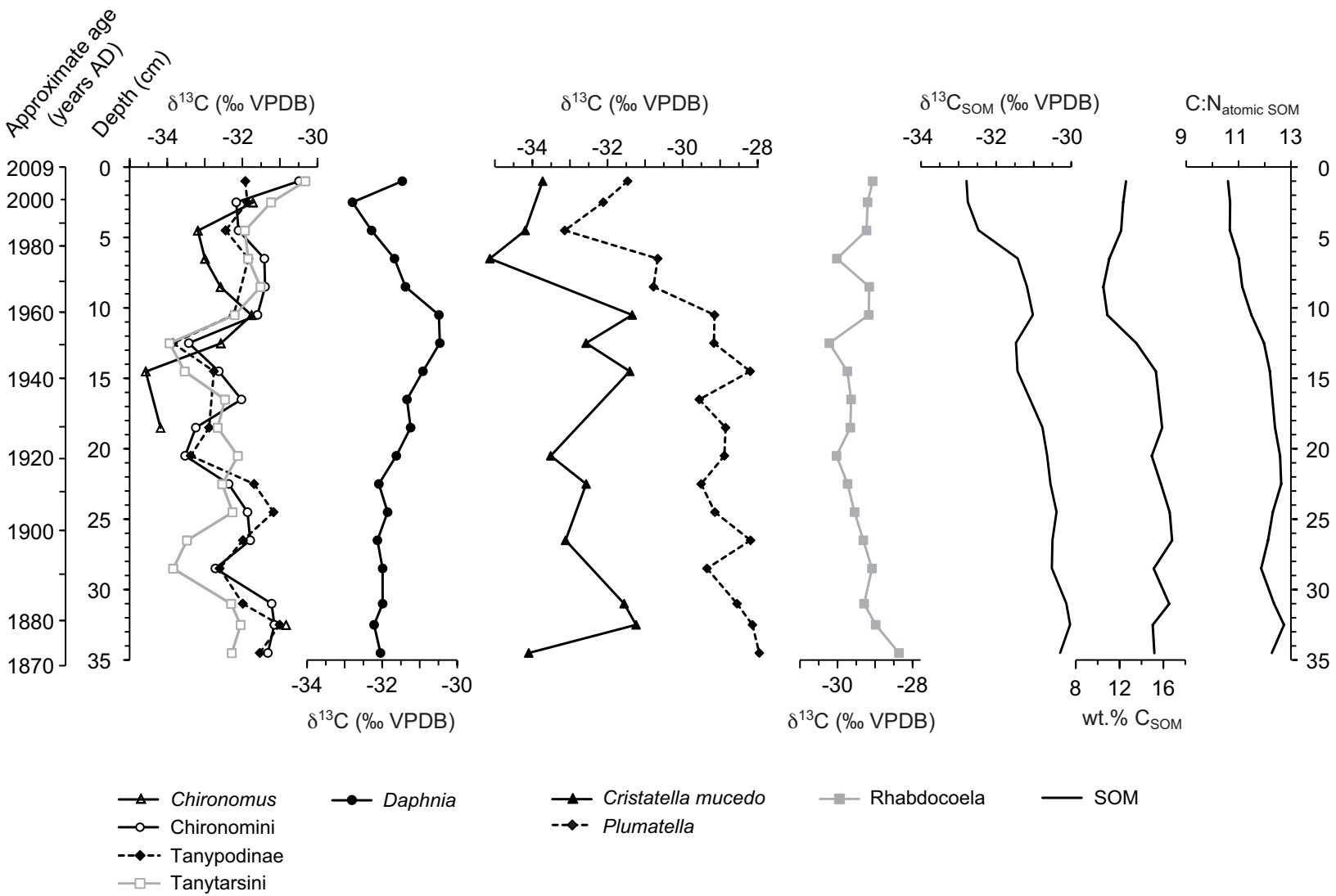


Figure 3
Click here to download Figure: Fig 3 Downcore Strandsjon v5.eps



Supplementary data for van Hardenbroek et al "Taxon-specific $\delta^{13}C$ analysis of chitinous invertebrate remains in sediments from Strandsjön, Sweden"

Supplementary table 1: $\delta^{13}C$, number of remains, and weight (in microgram) for each invertebrate sample. $\delta^{13}C$, $\delta^{15}N$, $\%C_{weight}$, $\%N_{weight}$, and $C:N_{atomic}$ ratio for sedimentary organic matter (SOM)

	Chironomus			Chironomini			Tanytarsini			Tanypodinae			*Daphnia*			*Cristatella mucedo*			*Plumatella*			Rhabdocoela			SOM				
Depth (cm)	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	$\delta^{13}C$	no.	mass	$\delta^{13}C$	$\delta^{15}N$	$\%C_{weight}$	$\%N_{weight}$	$C:N_{atomic}$
0-2				-30.53	21	18	-30.3	39	17	-31.9	15	10	-31.45	13	15	-33.7	1.25	29	-31.27	30	17	-29.1	40	36	-32.8	3.4	12.6	1.4	10.6
2-3	-31.7	19	18	-32.2	41	29	-31.2	41	20	-31.9	19	9	-32.8	31	28				-32.1	40	21	-29.2	40	37	-32.8	3.4	12.3	1.3	10.7
4-5	-33.2	27	24	-32.1	41	23	-31.9	47	15	-32.4	23	15	-32.3	32	29	-34.2	1	22	-33.1	40	29	-29.2	40	33	-32.5	3.4	12.2	1.3	10.7
6-7	-33.0	31	36	-31.4	33	22	-31.8	45	22	-31.8	22	15	-31.7	36	42	-35.1	1.25	43	-30.7	40	24	-30.0	40	40	-31.4	3.5	11.1	1.2	11.0
8-9	-32.6	31	41	-31.4	38	40	-31.5	45	28				-31.4	41	36				-30.8	45	21	-29.2	40	47	-31.2	3.5	10.5	1.1	11.1
10-11	-31.7	12	26	-31.6	31	29	-32.2	45	28	-32.2	31	27	-30.5	40	37	-31.3	1.5	59	-29.1	40	23	-29.2	40	48	-31.0	3.3	10.9	1.1	11.5
12-13	-32.6	8	11	-33.4	44	39	-33.9	47	32	-33.8	19	22	-30.5	41	34	-32.6	1.25	21	-29.2	40	24	-30.2	40	56	-31.5	2.8	13.6	1.3	12.0
14-15	-34.6	12	19	-32.6	16	12	-33.5	47	23	-32.8	20	17	-30.9	40	29	-31.4	1.5	34	-28.2	40	18	-29.7	40	48	-31.4	2.6	15.3	1.5	12.2
16-17				-32.0	38	38	-32.5	35	16				-31.3	30	44				-29.6	40	16	-29.6	40	47					
18-19	-34.2	15	24	-33.2	38	36	-32.7	50	21	-32.9	25	21	-31.2	41	54				-28.9	45	18	-29.7	40	53	-30.8	2.4	15.9	1.5	12.4
20-21				-33.5	39	42	-32.1	45	25	-33.4	33	27	-31.6	31	49	-33.5	2	34	-28.9	35	15	-30.0	40	51	-30.6	2.7	15.0	1.4	13.4
22-23				-32.4	35	42	-32.5	46	27	-31.7	14	15	-32.1	32	48	-32.6	1.25	22	-29.5	40	15	-29.7	40	61	-30.6	2.7	15.8	1.5	12.0
24-25				-31.9	39	42	-32.3	45	23	-31.2	15	20	-31.9	29	40				-29.1	40	13	-29.5	40	53	-30.4	2.6	16.3	1.5	12.0
26-27				-31.8	40	49	-33.5	45	29	-32.0	19	27	-32.1	30	39	-33.1	1	20	-28.2	42	15	-29.3	40	53	-30.5	2.6	16.6	1.6	11.9
28-29				-32.7	38	30	-33.8	45	24	-32.6	26	33	-32.0	31	48				-29.4	40	17	-29.1	40	46	-30.5	2.4	18.9	1.9	10.4
30-32				-31.2	38	28	-32.3	45	21	-32.0	24	30	-32.0	31	42	-31.6	2	17	-28.6	40	13	-29.3	40	53	-30.1	2.4	20.0	1.9	11.7
32-33	-30.8	8	9	-31.1	31	32	-32.0	45	25	-31.0	18	26	-32.2	30	39	-31.2	0.5	10	-28.1	40	14	-29.0	40	44	-30.0	2.3	20.1	1.8	12.7
34-35				-31.3	37	23	-32.3	45	24	-31.5	25	32	-32.0	30	34	-34.1	1	21	-28.0	42	16	-28.4	40	38	-30.3	2.3	19.1	1.8	12.9

Below recommended weight of 20 microgram